DOT BLOT PROTOCOL

For quick screening of elution fractions determine approximate protein amounts.

MATERIALS:

Protein samples (Elution fractions and flow-through), Control samples (HER2 + lysate), Nitrocellulose membranes (or other membrane), primary antibodies (mouse anti-V5 and human anti-HER2), secondary antibodies (Goat anti-mouse-HRP and anti-human-HRP), BioRad-imaging equipment, HRP reagents (Pierce™ ECL Western Blotting Substrate), blocking buffer (5 % milk in 1xTBST), 1xTBST buffer.

METHOD:

- Prepare dilutions of samples and controls. Dilute in MilliQ.
- Pippet 5 µl of dilutions onto the nitrocellulose membrane.

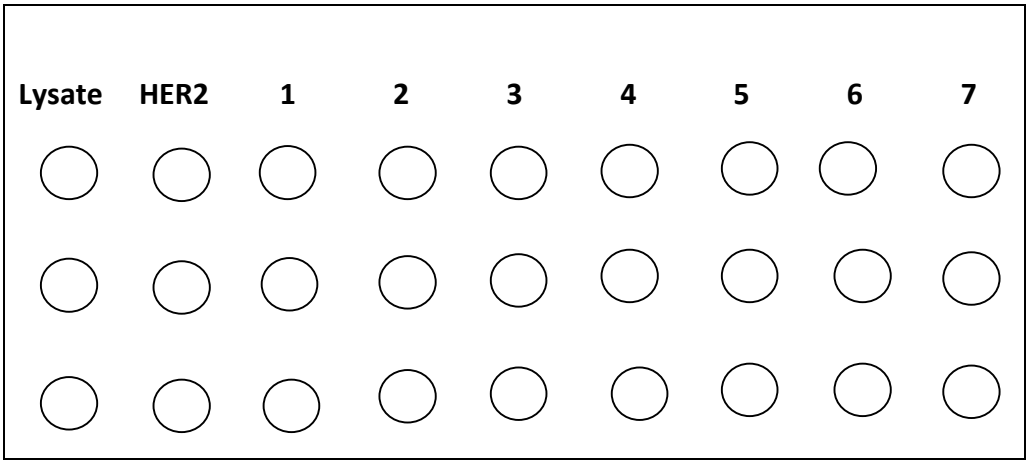

Figure 1: Dot blot sugestion for seven elution fractions. Each sample has three diltuions.

- Let the membrane dry in roomtemperature (RT).
- Block the membrane using cold blocking buffer. Incubate in RT for 1 h. Pour of buffer.
- Wash three times in TBST for 10 min eash.
- Apply primary antibody (diluted in blocking buffer or PBS) and incubate in RT for 1h or at 4°C overnight. Collect antibody solution.
- Wash three times in TBST for 10 min eash.
- Apply secondary antibody (diluted in blocking buffer) and incubate in RT for 1h. Collect antibody solution.
- Wash three times in TBST for 10 min eash.

- Mix HRP substrate reagents 1:1 and apply to membrane and incubate for 1 min in RT.
- Let the membrane dry sligthly on a tissue and transfer the membrane to a plastic sleeve for imaging.